

The solid fat content of stearic acid–rich fats determines their postprandial effects^{1–4}

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ABSTRACT

Background: The process of randomization is used commercially to harden fats as an alternative to partial hydrogenation, but its effects on cardiovascular disease risk factors are uncertain.

Objective: The objective was to compare the chronic and acute effects of randomization of a fat rich in 1,3-distearyl, 2-oleyl glycerol on fasting and postprandial lipids, glucose, insulin, and activated clotting factor VII (FVIIa) concentrations.

Design: A crossover design study in 16 men compared fasting and postprandial lipid, glucose, insulin, and FVIIa concentrations at baseline and after a 3-wk diet providing 30 g unrandomized or randomized shea butter and sunflower oil blends (SSOBs), both of which contained ≈50% stearic acid. Fecal fat excretion was measured during each dietary period. Postprandial changes were assessed after the consumption of meals providing 50 g test fat. A subsequent study compared postprandial changes after the consumption of an oleic acid–rich sunflower oil meal and an unrandomized SSOB meal.

Results: Both SSOBs were well digested and absorbed. Randomization did not affect fasting or postprandial lipid, glucose, insulin, or FVIIa concentrations. Compared with the oleic acid–rich meal, the unrandomized SSOB resulted in 53% lower postprandial lipemia, 23% higher hepatic lipase activity, and a 25% lower postprandial increase in FVIIa concentration. The solid fat contents at 37 °C were 22%, 41%, and 0% with the unrandomized SSOB, randomized SSOB, and oleic acid–rich meals, respectively.

Conclusions: Stearic acid–rich triacylglycerol in both unrandomized and randomized forms does not adversely affect lipid risk factors for cardiovascular disease. The high proportion of solid fat at 37 °C may explain the decreased postprandial lipemic response. *Am J Clin Nutr* 2007;85:1486–94.

KEY WORDS Stearic acid, postprandial lipemia, factor VII, triacylglycerol structure, fat digestion, physical properties of fats, randomization

INTRODUCTION

The process of random interesterification (randomization) of fats is widely being adopted by the food industry as an alternative to partial hydrogenation for the generation of fats with higher melting points. Saturated fatty acids (SFAs) in most vegetable fats are located in the outer positions of the triacylglycerol molecule (the *sn*-1 and *sn*-3 positions). Randomization of vegetable fats, therefore, leads to the generation of fats with a higher proportion of SFAs in the *sn*-2 position, similar to that of animal fats

such as lard. The physical changes that occur in fats after randomization are partly explained by the generation of trisaturated triacylglycerols but also by changes in the crystalline structure of fats that result from the different polymorphic forms of triacylglycerols, such as 1,3-distearyl, 2-oleyl glycerol (SOS). The different polymorphic forms, α , β^1 , and β , have melting points of 22.4, 36.5, and 41.6 °C, respectively (1).

Stearic acid may be better absorbed when situated in the *sn*-2 position of the triacylglycerol and, therefore, as 2-monoacylglycerol, than when situated in the *sn*-1 or *sn*-3 position because of the specificity of digestive lipases for the *sn*-1 and *sn*-3 positions (2, 3) and the tendency for free stearic acid to form insoluble calcium and magnesium soaps, which are poorly absorbed. Stearic acid in the *sn*-2 position of the dietary triacylglycerol is retained on absorption (4), and it has been suggested that stearic acid in the *sn*-2 position of the triacylglycerol may delay chylomicron clearance (5). These findings imply that triacylglycerol with stearic acid in the *sn*-2 position would be absorbed more efficiently but cleared from the circulation more slowly, which results in a more pronounced and prolonged postprandial lipemic response, than triacylglycerol with stearic acid in the *sn*-1 and *sn*-3 positions. This view is not supported by a study that compared 1,2 di-oleyl-stearyl glycerol (OOS) and 1,3 di-oleyl-stearyl glycerol (OSO) (6), but this finding may not be relevant for triacylglycerols containing 2 molecules of stearic acid. Furthermore, randomized stearic acid–rich triacylglycerol, which has a high proportion of stearic acid in the *sn*-2 position, has been reported to decrease postprandial lipemia and increase activated clotting factor VII (FVIIa) concentrations compared with oleic acid–rich triacylglycerol (7, 8) or cocoa butter, which has a unique triacylglycerol structure in which almost all of the

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stearic acid is present in the *sn*-1 and *sn*-3 position of the triacylglycerol (8, 9). In addition to changing the metabolic properties of triacylglycerol, randomization also results in changes in the physical properties of fats. Therefore, it remains unclear whether the differences in postprandial responses observed between fats with different triacylglycerol structures are due to their positional composition or are a consequence of changes in the physical properties of the fats (10). It is also possible that the process of randomization affects the digestibility of fat, which may partly explain the lower postprandial and FVIIa responses observed in previous studies.

To address these uncertainties, the aim of the present study was to compare the chronic and acute effects of unrandomized shea butter, which consists almost entirely of the triacylglycerol species SOS, with those of randomized shea butter, on lipid metabolism, FVIIa concentrations, and fat digestibility. In view of the results, a subsequent study compared the postprandial response to unrandomized shea butter with that to high-oleic acid sunflower oil, which is known to produce a similar level of lipemia as cocoa butter (8) and has a low melting point. The physical properties of the test fats were also determined. It was hypothesized that randomization of shea butter would result in a reduction in postprandial lipemia and FVIIa concentrations and that unrandomized shea butter would produce similar postprandial lipemia and an increase in FVIIa concentrations when compared with high-oleic acid sunflower oil.

SUBJECTS AND METHODS

Subjects

Twenty healthy male subjects were recruited from among staff and students of King's College London, University of London. The exclusion criteria included a history of cardiovascular disease, diabetes, a body mass index (in kg/m²) <20 or >35, plasma cholesterol >7.8 mmol/L, plasma triacylglycerol >3 mmol/L, current use of antihypertensive or lipid-lowering medication, and a self-reported intake of alcohol of >28 U/wk (1 U = 10 mL ethanol). Fasting plasma lipoprotein concentrations, body weight, blood pressure, blood cell count, and liver function were confirmed to be within the prescribed limits before entry into the study. Habitual nutrient intake was assessed from a 3-d food intake diary, completed during the 3-wk run-in period, and nutrient intakes were estimated by using the MICRODIET program (Downlee Systems Limited, Derbyshire, United Kingdom). The mean (±SD) daily energy intake was 7.8 ± 1.5 MJ; the percentage of energy from protein, carbohydrate, and fat was 15.9 ± 3.0%, 46.1 ± 9.4%, and 31.7 ± 6.5%, respectively. Four subjects withdrew from the first study after the first postprandial test: 1 because of self-reported gastrointestinal upset after the test meal, 2 because of time constraints, and 1 because of influenza. A total of 16 subjects completed the first study, 13 of whom took part in the follow-up study. Characteristics of the subjects at the time of screening are shown in **Table 1**.

Study design

A randomized crossover study design was used, which is summarized in **Figure 1**. The subjects followed a 3-wk run-in period with a low-stearic acid diet, during which time they were asked to avoid stearic acid-rich foods. At the end of the low stearic acid run-in period, the subjects were randomly allocated to consume

TABLE 1
Subject characteristics at screening¹

	Value
Age (y)	26.8 ± 8.0
Weight (kg)	74.8 ± 10.9
BMI (kg/m ²)	23.7 ± 3.7
Serum cholesterol (mmol/L)	4.5 ± 1.1
Serum LDL cholesterol (mmol/L)	2.6 ± 1.0
Serum HDL cholesterol (mmol/L)	1.5 ± 0.3
Serum triacylglycerol (mmol/L)	0.9 ± 0.5

¹ All values are $\bar{x} \pm SD$; $n = 16$.

1 of the 2 test fats (unrandomized or randomized stearic acid-rich fat). Fasting blood samples were collected, a test meal containing 50 g test fat was given, and postprandial blood samples were collected. The subjects then commenced the high-stearic acid dietary period, which required the daily consumption of 30 g test fat (consisting of the same fat that was in the test meal), which was provided as 2 small muffins for 3 wk. Toward the end of this period, the subjects made a 3-d fecal collection. At the end of the period, additional fasting and postprandial blood samples were collected. After a 4-wk washout period with the low-stearic acid diet, the subjects were crossed over to receive the other test fat, and the procedures were repeated. The subjects then resumed their habitual diet, and a subsequent study was conducted after a 4-wk washout period; fasting and postprandial blood samples were collected after the consumption of test meals containing 50 g unrandomized stearic acid-rich fat or oleic acid-rich fat, which were separated by ≥1 wk.

Test fats

High-oleic acid sunflower oil was obtained from Anglia Oils Ltd (Hull, United Kingdom). Shea butter, a stearic acid-rich fat, was supplied by Britannia Food Ingredients (Goole, United Kingdom) and was refined and randomized by Unilever Research (Vlaardingen, Holland) and blended with a small amount of sunflower oil so that linoleic acid accounted for ≈10% of the fatty acids, similar to the level present in high-oleic acid sunflower oil. The proportion of each molecular species in the test fats was determined by HPLC (11).

Dietary intervention

During the 3-wk high-stearic acid dietary periods, the subjects consumed 2 small muffins per day, each of which contained 15 g test fat (unrandomized or randomized shea blend). The subjects were advised to follow their habitual diet and consume the muffins instead of their usual snack products to prevent weight gain. Each muffin was formulated to provide 0.9 MJ (215 kcal), 2 g protein, 19 g carbohydrate, and 15 g fat and consisted of 2.8 g corn flour, 8.3 g baking flour, 8.3 g sugar, 1.1 g baking powder, 11 g skim milk, 1.1 g egg white, 1.1 g flavoring (essence), and 15 g test fat. The muffins were made in a variety of flavors to make them more palatable for the subjects. The muffins were made in batches and stored at −20 °C.

Formulation of test meals

The test meal consisted of 2 muffins (each containing 25 g test fat) and a milkshake formulated to provide 3.57 MJ (853 kcal), 50 g fat, 15 g protein, and 89 g carbohydrate. The milkshake consisted of 220 mL skim milk and 15 g Nesquik milkshake mix



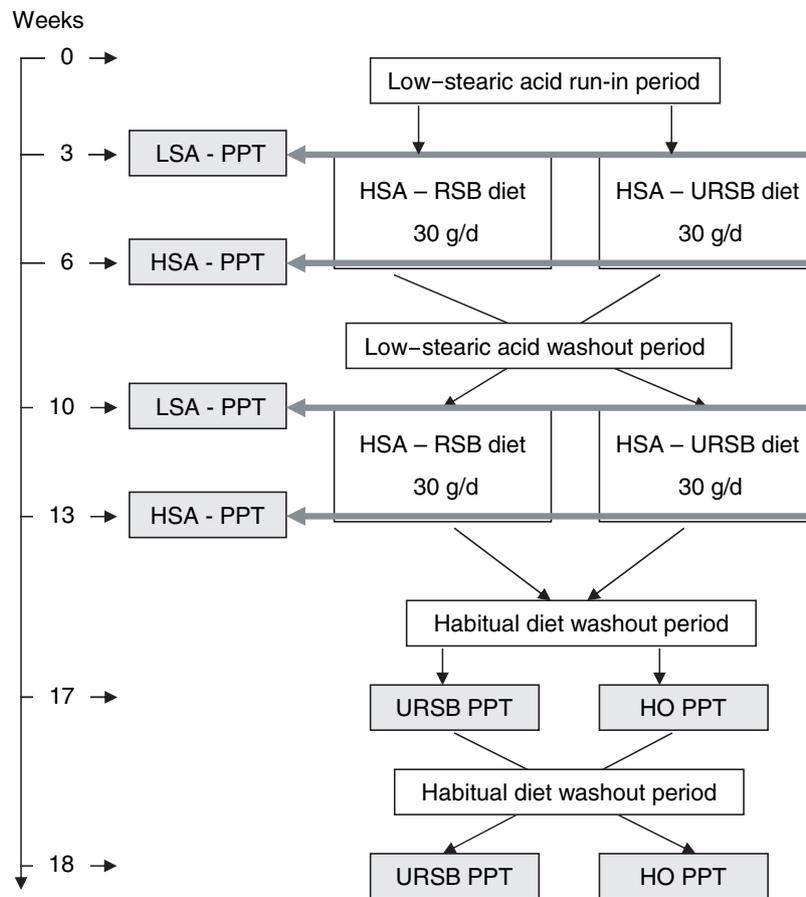


FIGURE 1. Study outline. PPT; postprandial test, LSA; low-stearic acid dietary period, HSA; high-stearic acid dietary period; RSB, randomized shea butter, URSB; unrandomized shea butter; HO, high-oleic acid sunflower oil.

(Nestlé Ltd, Switzerland). The 2 muffins contained 50 g test fat, 28 g baking flour, 10 g corn flour, 28 g sugar, 38 mL skim milk, 4 g pasteurized egg white, 4 g vanilla essence, and 2 g baking powder. For each study, the muffins were made in a single batch and stored at -20°C . Before being served, the muffins were defrosted and heated in a microwave until warm.

Postprandial test meal protocol

The day preceding each postprandial test, the subjects were advised to avoid consuming foods high in fat and were provided with a standardized low-fat dinner (containing <10 g fat) to consume as their evening meal. To control for physical activity levels, the subjects were asked to refrain from strenuous exercise, including cycling and sporting activities, and from the use of alcohol on the day before and on the day of the test meal. The subjects fasted overnight from 2200, and the following morning a cannula was inserted into the forearm (antecubital vein) of each subject between 0800 and 1000 to obtain fasting venous blood samples. The test meal was consumed within 15 min. In the main study, additional venous blood samples were obtained at 15 min, 30 min, 1 h and 90 min, and 2, 3, 4, 5, 6, 7, and 8 h. In the follow-up, study samples were collected at hourly intervals up to 6 h, after which the subjects received an intravenous injection of heparin (100 IU heparin/kg body weight; Monoparin, CP Pharmaceuticals Ltd, Wrexham, United Kingdom), and additional blood samples were collected 5 and 15 min later. During the postprandial period, the subjects refrained from the consumption

of any food or drink except water, which they were asked to consume at regular intervals throughout and after the 3-h blood sample was collected, after which the subjects received a standardized lunch (1.7 MJ) consisting of fresh fruit and low-fat yogurt (<1 g fat). This was previously shown not to interfere with the measurement of postprandial lipemia or the postprandial increase in FVIIa (7, 8). The study protocol was reviewed and approved by King's College Research Ethics Committee, and all participants gave written informed consent.

Collection and handling of blood samples

Venous blood samples were collected from a cannula into a syringe and dispensed into Vacutainers (Becton Dickinson, Plymouth, United Kingdom). Blood samples were processed within 30 min of blood collection. Blood for lipid analysis (plasma fatty acid and triacylglycerol concentrations) was collected into 6-mL EDTA-containing Vacutainers, and plasma was separated by centrifugation at $1500 \times g$ for 15 min at 4°C . Blood for lipoprotein analysis (total and HDL cholesterol) was collected into 2-mL Vacutainers containing no anticoagulant and was centrifuged at $1500 \times g$ for 15 min at 4°C . Blood for full blood counts was collected into 2-mL EDTA-containing Vacutainers for analysis on the same day. For FVIIa, blood was collected into 4.5-mL Vacutainers containing 38 g/L trisodium citrate solution and centrifuged at $1500 \times g$ for 15 min at 18°C . Three fasting blood samples were collected at 5-min intervals for glucose and insulin

analysis with the homeostasis model assessment of insulin resistance (HOMA-IR). Blood for glucose analysis was collected into 4-mL fluoride oxalate tubes, and blood for insulin analysis was collected into 2-mL lithium heparin-containing tubes; both samples were centrifuged at $1500 \times g$ for 15 min at 4 °C. EDTA samples (10 mL) for lipid analysis were collected at hourly intervals. Samples for lipoprotein measurement (2 mL, no anticoagulant), full blood counts (2 mL, EDTA), and FVIIa measurement (4.5 mL, trisodium citrate) were collected 3 and 6 h postprandially. The chylomicron-rich fraction [Svedberg flotation (S_f) >400] was separated by ultracentrifugation (from the EDTA plasma samples (6) at 2, 3, 4, and 5 h. In the main study, samples for glucose (4 mL fluoride oxalate) and insulin (2 mL lithium heparin) measurement were collected 15, 30, 60, 90, and 120 min postprandially. Blood for lipoprotein lipase (LPL) and hepatic lipase (HL) activity was collected into 6-mL lithium heparin-containing Vacutainers 5 and 15 min after the heparin injection and was centrifuged at $1500 \times g$ for 15 min at 4 °C.

Analytic methods

Melting characteristics of the tests fats were determined by differential scanning calorimetry with the use of a Mettler-Toledo DSC 820 unit (Mettler-Toledo Ltd, Leicester, United Kingdom). The solid fat content profiles were measured by pulsed nuclear magnetic resonance (NMR) analysis with the use of a QP²⁰⁺ pulsed NMR (Oxford Instruments Ltd, Oxfordshire, United Kingdom) at 5 temperatures (32, 37, 42, 47, and 52 °C). A standard preconditioning procedure for fats showing polymorphisms was followed; samples were melted at 80 °C and kept at 60 °C for 5 min followed by storage in a water bath at 26 °C for 40 h. Before the analysis was conducted, samples were equilibrated for 90 min at 0 °C and at each measurement temperature for 60 min.

Plasma total and HDL cholesterol and triacylglycerol concentrations were measured by enzymatic assays (8). Plasma insulin was measured with the use of a solid-phase, 2-site chemiluminescent immunometric assay on a DPC IMMULITE (Diagnostic Products Corporation, Caernarfon, United Kingdom). Plasma glucose was analyzed by using a full enzymatic colorimetric procedure (GOD-PAP) with an automated instrument (Boehringer Hitachi 704 EC; Boehringer Mannheim, Lewes, United Kingdom). Plasma FVIIa was measured by using a one-stage clotting assay as previously described (12). Plasma concentrations of palmitic, stearic, oleic, and linoleic acids were measured by gas-liquid chromatography (GLC) with pentadecanoic acid (15:0) as an internal standard (13) on a BP75 column (25 m \times 220 μ m \times 0.25 μ m; SGE Analytical Science Ltd, Milton Keynes, United Kingdom) on an Agilent 6890 (Agilent Technologies, Cheshire, United Kingdom). For determination of chylomicron triacylglycerol composition, lipids were extracted from the chylomicrons and the triacylglycerol fraction isolated by thin-layer chromatography and analyzed by GLC as described elsewhere (9). The composition of the fatty acids in the *sn*-2 position of the test fats and the chylomicron triacylglycerol fraction was determined by specific enzymatic hydrolysis (6) followed by separation of the 2-monoacylglycerol (2-MAG) by thin-layer chromatography and analysis of their fatty acid methyl esters by GLC.

LPL and HL activity were determined by using an adaptation of the continuous fluorimetric lipase test (CONFLOULIP; Progen Biotechnik, Heidelberg, Germany) on a Cobas Fara II with a

fluorescence measurement capability, fitted with a 400-nm emission filter, and a thermostatically controlled cuvette holder (37 °C). Two CONFLOULIP kits were used; the Total Lipase Test Kit and the Hepatic Lipase Select Test Kit. The modification from the kit method was to use high and low salt concentration buffers, both with a pH of 7.4. At low salt concentrations, LPL and HL are active and total lipase activity is measured; at high salt concentrations, LPL activity is inhibited so that only HL is active. LPL was then calculated by subtracting HL from total lipase activity.

Fecal samples were frozen within 12 h of collection. Three-day samples were pooled for each subject, defrosted, weighed, and homogenized; 100 g of the homogenate was freeze-dried and ground to a fine uniform powder. Total fat and fatty acid composition (g/d) were determined by using a modification of the method of Lepage and Roy (13) with heptadecanoic acid (17:0) as the internal standard and toluene instead of benzene as solvent.

Statistical analysis

Data that were not normally distributed were log transformed before analysis. Incremental area under the curves were calculated by using GRAPHPAD PRISM (version 3.0; GraphPad Software, Inc, San Diego, CA) using the trapezoid rule. Postprandial changes in plasma triacylglycerol and FVIIa were analyzed as the deviations from fasting on a log scale. Postprandial data for the main study are presented as values after the high-stearic acid diet, adjusted for values after the previous low-stearic acid diet period. Data were analyzed by using the repeated-measures analysis of variance (ANOVA) module of SPSS PC version 10 (SPSS Inc, Chicago, IL). For the main study, factors in the analysis were stearic acid concentration (values following the low-stearic acid diet versus values following the 3 wk high stearic acid diet), shea blend type (test meal consisting of randomized compared with unrandomized shea blends), and postprandial time points. In the follow-up study, factors were meal type (unrandomized shea blend compared with high-oleic acid sunflower oil) and postprandial time points.

RESULTS

The fatty acid composition, molecular species of triacylglycerol, and physical characteristics of the shea and sunflower oil blends are shown in **Table 2**. The unrandomized shea blend consisted mainly (76.2 mol%) of the triacylglycerol species SOS, whereas the proportion in the randomized shea blend was 46.3 mol%. The proportion of di- and trisaturated acyl glycerols increased from 6.7 and 2.7 mol% in the unrandomized shea blend to 21.5% and 10.1% in the randomized shea blend, respectively. Differential scanning calorimetry showed a major melting peak at 40 °C for the unrandomized shea blend and 2 major melting peaks for the randomized shea blend at 44 and 55 °C. Low-resolution NMR measurements indicated that the unrandomized shea blend had a negligible solid fat content at temperatures >42 °C compared with 23% for the randomized shea blend; at 37 °C, it was 22.2% compared with 41.2% for the randomized shea blend. For comparison, the high-oleic acid sunflower oil contained 83.9 mol% oleic acid, 8.0 mol% linoleic acid, and 1.6 mol% stearic acid, and the major triacylglycerol was triolein; all of the fat was melted at approximately -8 °C.

There were no side effects or abnormal stool habits associated with consumption of the randomized and unrandomized shea

TABLE 2Fatty acid and triacylglycerol composition and solid fat content of randomized and unrandomized shea blends¹

	Randomized shea blend	Unrandomized shea blend
Triacylglycerols (mol%)		
SSS	10.1	2.7
SOS	46.3	76.2
SSO	15.8	< 0.1
SLS	4.3	6.5
SSL	1.4	< 0.1
SOO	12.4	10.2
OSO	3.6	< 0.1
SLO/SOL/OSL	2.4	1.8
OOO	2.6	1.7
Others	1.1	0.9
Fatty acid composition (mol%)		
16:0 (palmitic)	3.3	2.9
18:0 (stearic)	49.8	53.3
18:1n-9 (oleic)	34.9	33.3
18:2n-6 (linoleic)	10.3	8.9
Others	1.8	1.7
Solid fat content (%)		
32 °C	56.5	57.2
37 °C	41.2	22.2
42 °C	23	< 1.0
47 °C	15.2	< 1.0
52 °C	10	< 1.0

¹ O, oleic acid; P, palmitic acid; S, stearic acid; L, linoleic acid.

blends reported by subjects. Compared with the low-stearic acid diet, the proportion of stearic acid in fasting total plasma lipids increased to a similar amount (by 24%) after both the randomized and unrandomized high-stearic acid diets, indicating that both types of fat had similar bioavailability (**Table 3**). Fecal fat excretion ($\bar{x} \pm SD$) was low with the high-stearic acid diet and did not differ after the randomized (3.9 ± 2.6 g/d; range: 0.5–10.1 g/d) and unrandomized (3.5 ± 1.7 g/d; range: 0.3–6.7 g/d) shea

TABLE 3Fasting lipid, glucose, and insulin concentrations and homeostatic model assessment for insulin resistance (HOMA-IR) after the low- and high-stearic acid diets¹

	Randomized shea blend		Unrandomized shea blend	
	Low stearic acid	High stearic acid	Low stearic acid	High stearic acid
Total cholesterol (mmol/L)	4.09 \pm 0.98 ²	4.26 \pm 0.91	4.32 \pm 0.96	4.23 \pm 0.88
HDL cholesterol (mmol/L)	1.23 \pm 0.24	1.30 \pm 0.22	1.28 \pm 0.21	1.28 \pm 0.22
LDL cholesterol (mmol/L)	2.41 \pm 0.89	2.42 \pm 0.77	2.51 \pm 0.87	2.42 \pm 0.83
Triacylglycerol (mmol/L)	0.93 (0.69, 1.26) ³	0.98 (0.77, 1.26)	0.90 (0.67, 1.20)	0.92 (0.69, 1.22)
IAUC triacylglycerol (arbitrary units)	123 (65, 234)	143 (100, 205)	116 (70, 193)	117 (69, 200)
Plasma stearic acid (% by wt of total fatty acids) ⁴	7.02 (6.51, 7.58)	8.75 (8.14, 9.39)	7.11 (6.68, 7.56)	8.76 (7.80, 9.83)
Glucose (mmol/L)	5.33 \pm 0.48	5.44 \pm 0.47	5.22 \pm 0.52	5.44 \pm 0.45
Insulin (mU/L) ⁵	6.56 (5.73, 7.50)	6.90 (5.05, 9.41)	6.73 (5.58, 8.13)	6.82 (5.66, 8.22)
HOMA-IR ⁵	1.6 \pm 0.6	1.9 \pm 1.6	1.6 \pm 0.5	1.8 \pm 0.6
Weight (kg)	75.0 \pm 10.9	75.2 \pm 10.7	75.2 \pm 10.8	75.2 \pm 10.6

¹ $n = 16$. IAUC, incremental area under the curve. Data were analyzed by ANOVA with shea blend (unrandomized compared with randomized) and dietary stearic acid content (low compared with high) as factors; there were no significant shea blend \times dietary stearic acid interactions. Values did not differ significantly between groups after the low-stearic acid diet.

² $\bar{x} \pm SD$ (all such values).³ Geometric \bar{x} ; 95% CI in parentheses (all such values).⁴ Significant effect of dietary stearic acid content (low compared with high), $P < 0.001$.⁵ $n = 13$.

diets. Stearic acid accounted for half of the fecal fat, but there were no differences between the randomized ($50.9 \pm 16.0\%$ by wt) and unrandomized ($49.4 \pm 13.0\%$ by wt) shea diets.

After the high-stearic acid diets, fasting total, LDL, and HDL cholesterol; plasma triacylglycerol, glucose, and insulin concentrations; and HOMA-IR did not differ significantly from values after the low-stearic acid diet or between randomized and unrandomized shea blends (**Table 3**).

Postprandial measurements

There were no significant differences in postprandial responses between the low- and high-stearic acid diets; no significant differences were observed in full blood counts and postprandial measures of plasma triacylglycerol, plasma fatty acid, chylomicron triacylglycerol fatty acid, insulin, glucose, serum cholesterol, and FVIIa concentrations, as measured by ANOVA of shea blend (randomized compared with unrandomized) \times time (h) \times dietary stearic acid content (low compared with high) factors. Postprandial changes in plasma triacylglycerol after the test meals at the end of each high-stearic acid dietary intervention period are shown in **Figure 2**. The maximum increase occurred at 4 h: 62.7% (95% CI: 23.4, 114.9) for the unrandomized shea blend and 76.3% (95% CI: 55.3, 100.4) for the randomized shea blend. Values were higher 8 h after the randomized shea blend than after the unrandomized shea blend ($P = 0.007$).

The postprandial increases in total fatty acid and plasma stearic, palmitic, oleic, and linoleic acid concentrations did not differ between unrandomized and randomized shea blends (data not shown). Similarly, there were no significant differences in the proportions of fatty acids in the chylomicron triacylglycerol between shea blends (mean of values from 2 to 6 h), as shown in **Table 4**. The proportion of palmitic, oleic, and linoleic acids in the chylomicron triacylglycerol largely reflected those in the shea blends; however, stearic acid was significantly lower in the chylomicron triacylglycerol after the randomized (mean of values from 2 to 6 h: 35.9 mol%; 95% CI: 33.8, 37.9) and unrandomized (mean of values from 2 to 6 h: 36.2 mol%; 95% CI: 35.0,

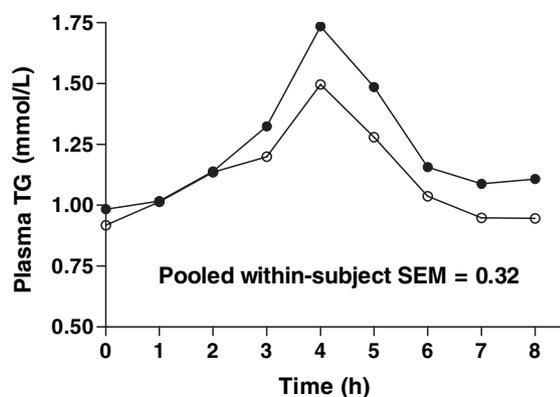


FIGURE 2. Geometric mean plasma triacylglycerol (TG) concentrations after test meals containing 50 g unrandomized (○) and 50 g randomized (●) shea blends in 16 male subjects after 3 wk of a high–stearic acid diet containing 30 g shea blend/d, adjusted for values after consumption of the previous low–stearic acid diet. Data were log transformed, and the deviations from fasting were analyzed by repeated-measures ANOVA with shea blend (2 meals: unrandomized compared with randomized), time (8 time points), and dietary stearic acid content (2 levels: low compared with high) as factors: the time effect was significant ($P < 0.001$), and the shea blend \times time interaction and shea blend \times time \times dietary stearic acid interactions were not statistically significant.

37.5) shea blend compared with the shea blends consumed (randomized and unrandomized shea blend; 49.8 and 53.3 mol%, respectively) at all time points (h). The distribution of fatty acids in the *sn*-2 position of the chylomicrons broadly reflected that of the shea blends (Table 4), and there was a significantly ($P = 0.031$) higher proportion of stearic acid in the *sn*-2 position in the chylomicrons after the randomized shea blend than after the unrandomized shea blend. No differences were observed in postprandial serum cholesterol (total, HDL, and LDL cholesterol), glucose, or insulin concentrations after the randomized or unrandomized shea blends (data not shown).

In the follow-up study, plasma triacylglycerol concentrations increased to a lesser extent after the unrandomized shea blend than after the high–oleic acid sunflower oil, but the pattern of response was not significantly different between meals (Figure 3). Repeated-measures ANOVA of meal (unrandomized shea blend compared with high–oleic acid sunflower oil) \times time (6

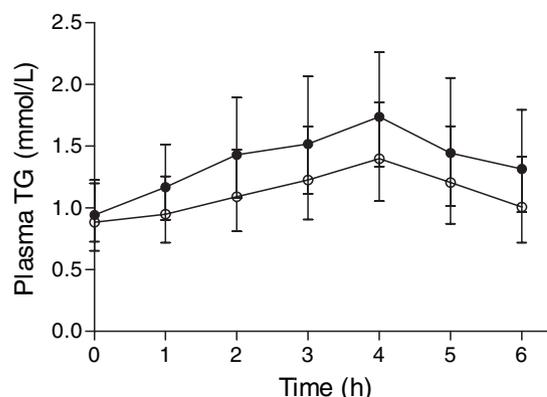


FIGURE 3. Geometric mean (95% CI) plasma triacylglycerol (TG) concentrations after test meals containing 50 g unrandomized shea blend (○) and 50 g high–oleic acid sunflower oil (●) in 13 male subjects. Data were log transformed, and the deviations from fasting were analyzed by ANOVA with meal (2 meals: unrandomized shea blend compared with high–oleic acid sunflower oil) and time (6 time points) as factors: the meal effect was significant ($P = 0.035$), the time effect was significant ($P < 0.001$), but the meal \times time interaction was not significant.

factors showed a significant time effect for triacylglycerol concentrations ($P < 0.001$) and a significant time and meal effect for deviations from fasting ($P < 0.001$ and $P = 0.035$, respectively). The incremental area under the curve for plasma triacylglycerol was higher ($P = 0.017$) after the high–oleic acid sunflower oil than after the unrandomized shea blend. Peak 4-h increases in plasma triacylglycerol concentrations after the unrandomized shea blend were 58% (95% CI: 27, 98) in the follow-up study compared with 63% (95% CI: 23, 115) in the main study, which showed good reproducibility in postprandial responses between studies.

Concentrations of plasma stearic and oleic acids were significantly different between the shea blend and the high–oleic acid sunflower oil (meal \times time interaction; $P < 0.001$ for both), and large increments from fasting values in stearic acid (peak increase of 108% at 5 h; 95% CI: 69, 155) were observed after the unrandomized shea blend and large increases in oleic acid (peak increase at 4 h of 102% (95% CI: 77, 131) were observed after the high–oleic acid sunflower oil (data not shown). The proportion

TABLE 4

Postprandial fatty acid composition of the venous chylomicron triacylglycerol (TG) and proportions of fatty acids in the *sn*-2 position of the postprandial chylomicron TG and shea blends consumed¹

Fatty acid	Chylomicron TG ²		<i>sn</i> -2 Position in shea blend		<i>sn</i> -2 Position in chylomicron TG ³	
	Randomized shea blend	Unrandomized shea blend	Randomized shea blend	Unrandomized shea blend	Randomized shea blend	Unrandomized shea blend
	mol%		mol%		mol%	
16:0	10.8 (9.4, 12.2)	10.2 (9.3, 11.1)	1.7	0.6	8.1 (2.0, 14.2)	7.7 (5.6, 9.8)
18:0	35.9 (33.8, 37.9)	36.2 (35.0, 37.5)	22.8	3.1	19.6 (14.3, 24.9) ⁴	11.0 (2.8, 19.1)
18:1n-9	41.1 (40.2, 42.0)	41.5 (40.6, 42.3)	61.1	78.8	56.4 (51.0, 61.7)	65.8 (56.3, 75.3)
18:2n-6	12.2 (11.6, 12.9)	12.1 (11.8, 12.4)	13.5	17.3	16.6 (14.3, 19.0)	15.3 (13.3, 17.3)

¹ Data were analyzed by ANOVA with shea blend (unrandomized compared with randomized) and dietary stearic acid content (low compared with high) as factors; there were no significant shea blend \times dietary stearic acid interactions.

² All values are means; 95% CIs in parentheses. $n = 16$ (mean of values from 2 to 6 h adjusted for values after the previous low–stearic acid diet).

³ All values are means; 95% CIs in parentheses. $n = 4$ (mean of values from 2 to 6 h adjusted for values after the previous low–stearic acid diet; 4 pools of 4 subjects for each).

⁴ Significantly different from unrandomized shea blend, $P = 0.031$ (paired t test).

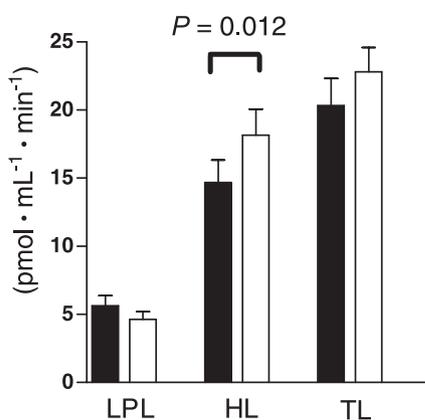


FIGURE 4. Mean (\pm SEM) plasma postheparin lipoprotein lipase (LPL), hepatic lipase (HL), and total lipase (TL) activities (of 5 and 15 min) in 13 male subjects 6 h after test meals containing 50 g high-oleic sunflower oil (■) and 50 g unrandomized shea blend (□). Comparisons between meals were made by using a paired *t* test.

of fatty acids in the chylomicron triacylglycerol (mean of values from 2 to 6 h) after the high-oleic sunflower oil reflected that of the test fat (data not shown). However, there was a significantly lower proportion of stearic acid in the chylomicron triacylglycerol after consumption of the unrandomized shea blend (mean of values from 2 to 6 h: 35.3 mol%; 95% CI: 33.2, 37.5) than after consumption of the test fat (53.3 mol%), which was similar to observations in the main study (mean of values from 2 to 6 h: 36.2 mol%; 95% CI: 35.0, 37.5). Total lipase, LPL, and HL activities are shown in **Figure 4**. HL activity was significantly higher (23%) after the unrandomized shea blend than after the high-oleic sunflower oil ($P = 0.012$). No significant differences were observed in postprandial serum cholesterol concentrations (total, HDL, and LDL cholesterol) after the unrandomized shea blend and the high-oleic acid sunflower oil (data not shown).

FVIIa concentrations did not differ significantly after the low- and high-stearic acid diets between randomized (geometric \bar{x} : 52.1 $\mu\text{g/L}$; 95% CI: 45.7, 59.3) and unrandomized (geometric \bar{x} : 48.6 $\mu\text{g/L}$; 95% CI: 38.6, 61.0) shea blends, and there was no significant change with time from fasting after either shea blend (**Figure 5A**). FVIIa increased significantly from fasting values after the high-oleic acid sunflower oil at 3 and 6 h ($P < 0.05$ and $P < 0.01$ respectively), but not after the unrandomized shea blend (**Figure 5B**). Fasting FVIIa values were as follows: after the unrandomized shea blend (geometric \bar{x} : 43.3 $\mu\text{g/L}$; 95% CI: 34.1, 55.0) and after the high-oleic acid sunflower oil (geometric \bar{x} : 56.9 $\mu\text{g/L}$; 95% CI: 45.2, 71.6). There was a significant meal effect ($P = 0.04$) when the deviations from fasting values were compared. Full blood counts did not differ postprandially between the test fats (for both randomized compared with unrandomized shea blends and for high-oleic acid sunflower oil compared with unrandomized shea blend; data not shown). However, in both studies, there was a significant time (h) effect for white blood cells ($P < 0.001$), neutrophils ($P < 0.001$), and lymphocytes ($P = 0.001$), which increased 3 and 6 h postprandially (**Figure 6, A and B**). No other significant differences were noted.

DISCUSSION

The aim of this study was to test the hypothesis that the randomization of a fat consisting mainly of the triacylglycerol species SOS would decrease postprandial lipemia and the associated

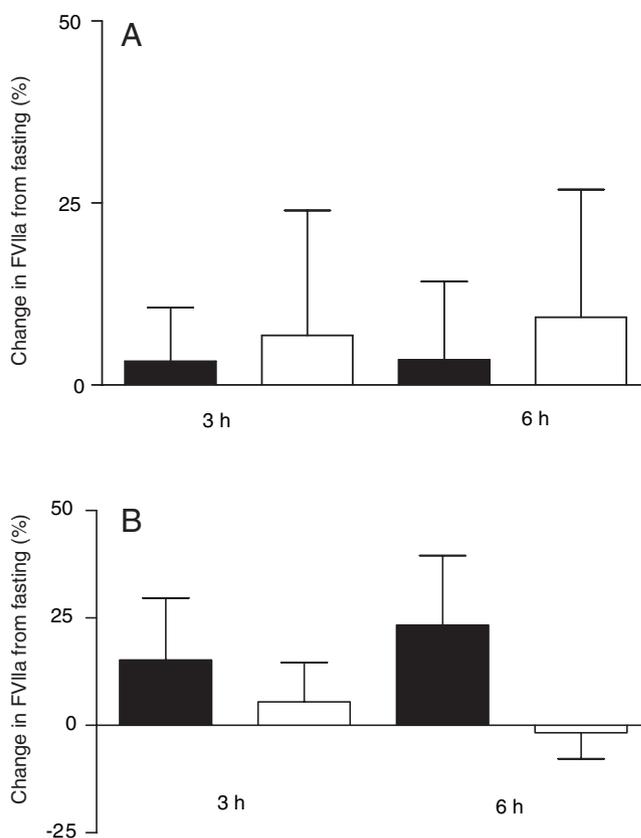


FIGURE 5. A: Change from fasting values (95% CI) in plasma activated clotting factor VII (FVIIa) concentrations after test meals containing 50 g unrandomized (□) and 50 g randomized (■) shea blends in 16 male subjects after 3 wk of a high-stearic acid diet providing 30 g shea blend/d, adjusted for values after the previous low-stearic acid diet. Data were log transformed, and the deviations from fasting were analyzed by repeated-measures ANOVA with shea blend (2 meals: unrandomized compared with randomized), time (2 time points), and dietary stearic acid content (2 levels: low compared with high) as factors: shea blend \times time interaction and shea blend \times time \times dietary stearic acid interactions were not statistically significant, and there were no significant changes with time. B: Change from fasting values (95% CI) in plasma activated clotting factor VII (FVIIa) concentrations after test meals containing 50 g unrandomized shea blend (□) or 50 g high-oleic acid sunflower oil (■) in 11 male subjects. Data were log transformed, and the deviations from fasting were analyzed by repeated-measures ANOVA with meal (2 meals) and time (2 time points) as factors: the meal effect was significant ($P = 0.04$), and the meal \times time interaction was not significant.

increase in FVIIa concentration. However, the results of the present study differ from those of previous studies, which used randomized cocoa butter, randomized short- and long-chain triacylglycerols, or a blend of randomized totally hydrogenated sunflower oil and unhydrogenated high-oleic acid sunflower oil.

In agreement with previous studies, the chronic consumption of these stearic acid-rich fats, which provided an additional 15 g stearic acid/d ($\approx 7\%$ of the energy intake), had no adverse effects on the lipid profile or on indexes of insulin sensitivity compared with the low-stearic acid background diet. This finding supports the view that stearic acid-rich fats should not be regarded as cholesterol raising (14).

As predicted, randomization resulted in differences in the proportions of stearic acid in the *sn*-2 position of the chylomicron triacylglycerol. It was surprising, therefore, that there were no

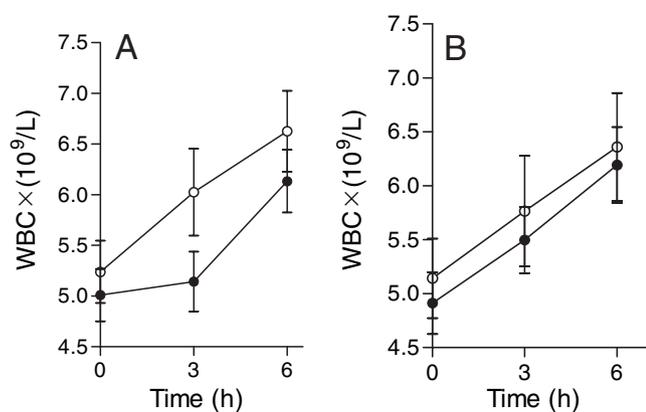


FIGURE 6. A: Mean (\pm SEM) white blood cell (WBC) counts after test meals containing 50 g unrandomized (\circ) and 50 g randomized (\bullet) shea blends in 16 male subjects after 3 wk of a high-stearic acid diet providing 30 g shea blend/d, adjusted for values after a previous low-stearic acid diet. Data were log transformed, and the deviations from fasting were analyzed by repeated-measures ANOVA with shea blend (2 meals: unrandomized compared with randomized), time (2 time points), and dietary stearic acid (2 levels: low compared with high) as factors: the time effect was significant ($P < 0.001$), but there was no significant effect of meal or dietary stearic acid content. B: Mean (\pm SEM) WBC counts after test meals containing 50 g unrandomized shea blend (\circ) and 50 g high-oleic acid sunflower oil (\bullet) in 13 male subjects. Data were analyzed by repeated-measures ANOVA with meal (2 meals) and time (2 time points) as factors: the time effect was significant ($P < 0.001$), but there was no significant effect of meal.

significant differences between the randomized and unrandomized shea blends on postprandial plasma triacylglycerol concentrations. However, comparison of the unrandomized shea blend with the high-oleic acid sunflower oil showed that it decreased lipemia and failed to increase FVIIa postprandially. This effect was unlikely to have been a consequence of malabsorption because fecal fat excretion remained within the normal range, and other reports indicate that both symmetrical unrandomized and randomized stearic acid-rich fats are well absorbed (15–18). Consequently, this would not explain the low postprandial response observed after both types of shea blends. Furthermore, the substantially higher proportion of fats with a high melting point in the randomized shea blend did not appear to have an adverse effect on absorption. Another possible explanation could be that the rates of absorption and chylomicron secretion are slower or the rate of clearance of these particles is accelerated with these stearic acid-rich fats without any significant decline in digestibility. The prolonged postprandial lipemia observed after the shea blends and the delayed appearance of stearic acid in the chylomicron triacylglycerol compared with oleic acid supports this view. A novel observation of this study, which requires confirmation, is that HL activity was greater after the unrandomized shea blend than after the high-oleic acid sunflower oil. This might indicate an adaptive response to cope with a lower substrate specificity for stearic acid-rich triacylglycerol in extrahepatic tissues compared with triolein. It could also indicate a faster rate of chylomicron removal from the circulation and partially explain the lower postprandial response after the shea blends. However, LPL activities were not significantly different between meals, an observation also reported by Tholstrup et al (19) after meals rich in oleic and stearic acids. Additional studies are needed to investigate the mechanisms involved.

Neither randomized nor unrandomized shea blends resulted in postprandial increases in FVIIa concentrations in contrast with

the significant increase observed after the consumption of high-oleic acid sunflower oil. This finding is consistent with that observed with other stearic acid-rich triacylglycerols, ie, decreased postprandial lipemia (7, 9). The mechanism responsible for the postprandial activation of FVII is unclear. Previous studies have not found a clear relation between postprandial lipemia measured as the area under the curve or peak lipemia and the extent of the postprandial increase in FVIIa. Activation of FVII may occur via interaction with a charged surface, such as those provided by membrane lipids. The large increase in FVIIa after the high-oleic acid meal may have been due to activation of the ABC-A1 transporter protein, which may be stimulated by unsaturated fatty acids, resulting in the expression of charged phospholipids on the outer surface of leukocytes and platelets (20). Because postprandial lipemia is accompanied by an increased outflow of lymph, we hypothesized that changes in postprandial leukocyte counts might explain the increase in FVIIa. However, leukocyte counts increased substantially after the test meals, regardless of their ability to increase FVIIa. Additional studies are required to examine the mechanisms leading to FVII activation.

To investigate the differences in postprandial lipemia, we analyzed the physical properties of the test fats. This investigation indicated that both shea blends had a high proportion of solid fat at 37 °C (randomized shea blend: 41%; unrandomized shea blend: 22%), whereas high-oleic sunflower oil had no solid fat at 37 °C. We subsequently undertook further NMR analysis of fats from previous studies by our group (10) and found that other stearic acid-rich fats, which resulted in lower lipemia, had a high solid fat content [37% and 23% solid fat at 37 °C for the randomized cocoa butter and randomized partially hydrogenated sunflower oil (7, 9), respectively, compared with the unrandomized fats (1% for cocoa butter)]. It is proposed that fats that contain crystalline solids at body temperature may affect micelle formation and retard the process of absorption and consequently result in reduced postprandial lipemia. These differences may have implications regarding the risk of cardiovascular disease. In conclusion, the present study indicated no adverse effects of randomized stearic acid-rich fats on cardiovascular disease risk factors.

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The authors' responsibilities were as follows—TABS and SEEB: conceived and devised the study and contributed to the analysis and writing of the manuscript; and GJM: supervised the FVIIa analysis. The authors had no financial or commercial interest in any company or organization involved with this study.

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